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Oxidative Effects of 1,1,3-Tricyano-2-Amino-1-Propene. (26695)

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In 1948 it was reported that a solution of malononitrile increased nucleic acid concentration of large nerve cells of the central nervous system of rabbits(1). Since other investigators were unable to reproduce this finding, a series of spectral analyses of fresh and aged solutions of the chemical was undertaken, indicating that active material was probably a decomposition product which formed on standing(2). Isolation and characterization(3) of 1,1,3-tricyano-2-amino-1-propene (U-9189) from similar aged solutions was reported to give the "nucleic acid effect" mentioned above and to uncouple oxidative phosphorylation *in vitro*(4). The compound was found to have antithyroid activity in humans and inhibit thyroxine formation in rats(5) as well as cause pyrexia in rats.* Because this suggested alteration in gross metabolism of treated animals, it was decided to quantitate effects on oxidation rates of various C^{14}-labeled compounds in rats.

Materials and methods. Adult male albino rats of Upjohn colony (Sprague-Dawley ancestry), weighing 175-180 g and fasted 18 hr (overnight), were used for respiratory studies. Fasted young male rats weighing 60-70 g were used for age comparison studies. Intraperitoneal doses of 50 mg/kg of U-9189 or 15 mg/kg of 2,4-dinitrophenol† (DNP) used in these studies produced slight fevers of 1 to 1.5°F for 1-3 hr in intact and thyroidectomized rats. Doses of U-9189 and DNP were made fresh daily in deionized water or 1/10 N sodium bicarbonate respectively; 1 ml volumes were injected. Some radioactive compounds were injected intraperitoneally as 0.5 ml aqueous solutions of: C^{14}-2-acetate, sodium (3.3×10^6 cpm, 0.45 mg), C^{14}-2-glycine (1×10^6 cpm, 0.17 mg), C^{14}-glucose (uniformly labeled, 1×10^6 cpm, 0.19 mg), and C^{14}-1-DL-leucine (8.8×10^5 cpm, 0.27 mg). C^{14}-tripalmitin carboxyl labeled) was incorporated into an emulsion similar to Lipomul‡ and 0.6 ml (2.4×10^5 cpm, 0.43 mg of C^{14}-1-tripalmitin) injected into a femoral vein.

In a typical experiment, rats were injected with either U-9189 or DNP and ½ hr later with the C^{14} compound and placed in glass metabolic units from which the respired CO_2 was collected. Exhaled $C^{14}O_2$ was counted as $BaC^{14}O_3$ by a procedure previously reported (6). In one series of rats, total respiratory CO_2 was evaluated by $BaCO_3$ collection, after which thyroid glands were removed. After 10 days of recovery, respiratory CO_2 was measured again and rats with 24-30% depression in CO_2 output were considered thyroidectomized and used in isotope experiments (Table IV).

Results and discussion. Oxidation rates by intact adult rats. Results (Table I) show that, ½ hr after injection of radioactivity, U-9189 increased oxidation rates of all C^{14}-labeled compounds except glucose. Compared to their respective controls, peak stimulation of acetate oxidation was 83% at ½ hr, tripalmitin 33% at 1 hr, DL-leucine 61% at 1 hr, and glycine 110% at ½ hr. In view of the fact that U-9189 caused an increased output of total CO_2 of about 34% during 5 hr

* Seay, P. H., McNeil Labs., Philadelphia, Pa., Unpublished data.

† Eastman Organic Chemicals, Rochester, N. Y.

‡ Upjohn trademark for intravenous cottonseed oil emulsion.

TABLE I. Survey of Effects of U-9189 on Cumulative Oxidation of Various Labeled Compounds by Fasting Rats.*

Group		% of inj. C^{14} oxidized to $C^{14}O_2$ at: ½ hr	1 hr	2 hr	3 hr	5 hr
C^{14}-2-Acetate	Control (5)†	14.0 ± 1.6§	29.4 ± 2.7	42.6 ± 3.1	48.4 ± 2.4	55.3 ± 2.1
	U-9189‡ (5)	25.6 ± 1.5	44.8 ± 2.4	52.3 ± 2.2	56.1 ± 3.2	65.2 ± 5.1
C^{14}-1-Tripalmitin	Control (6)	6.9 ± .4	19.3 ± .9	43.6 ± 3.3	57.1 ± 4.0	72.9 ± 4.6
	U-9189 (6)	9.0 ± .9	24.7 ± 1.9	51.6 ± 2.9	69.0 ± 5.9	80.6 ± 4.2
C^{14}-1-Leucine	Control (4)	5.5 ± .4	11.3 ± 1.1	18.6 ± .4	23.7 ± 1.2	27.3 ± 1.0
	U-9189 (4)	8.5 ± 2.6	18.2 ± 2.6	26.6 ± 2.7	30.2 ± 2.2	32.0 ± 1.9
C^{14}-2-Glycine	Control (5)	1.1 ± .2	2.5 ± .6	4.9 ± .8	6.6 ± .9	9.9 ± 1.1
	U-9189 (5)	2.3 ± .1	5.1 ± .2	8.5 ± .6	10.8 ± 1.3	17.0 ± 2.5
C^{14}-UL-Glucose	Control (4)	6.3 ± .5	18.6 ± 1.0	24.0 ± 1.5	37.9 ± 1.4	45.7 ± 1.7
	U-9189 (4)	5.7 ± .8	21.4 ± 1.6	27.7 ± 3.5	35.7 ± 4.0	45.2 ± 3.5

* All rats given U-9189 showed an increase of 34 ± 7% in total CO_2 exhaled by 5th hr.
† No. of rats used to obtain group mean.
‡ 1,1,3-tricyano-2-amino-1-propene.
§ Stand. error of mean.

of measurement (Table I footnote), specific activity changes (Table II) are more relevant in evaluating comparative effect of U-9189 on oxidative metabolism. For acetate, leucine, and glycine, increased C^{14} to C^{12} ratio was marked, suggesting increased utilization in excess of that produced by increased metabolic rate. In the case of glycine this was particularly sustained, while with tripalmitin there was a smaller but significant increase lasting less than an hour. Specific oxidation of glucose carbon was definitely less compared to controls, therefore other carbon sources of energy were utilized in greater proportion in presence of the drug.

Oxidative effects in young and adult rats. In young rats, 50 mg/kg of U-9189 or 15 mg/kg of DNP had little or no effect on oxidation of acetate (Table III). However, compared to adult rats, young control rats oxidized acetate considerably faster, a rate which apparently could not be further increased by administration of drugs.

TABLE II. Effect of U-9189 on Specific Activity of Total Expired CO_2 from Rats Given Various C^{14} Labeled Compounds.*

C^{14}-compound oxidized	Specific activity (% of control) at: ½ hr	1 hr	2 hr	3 hr	5 hr
C^{14}-2-Acetate	164 ± 10	117 ± 3	0†	0	0
C^{14}-1-Tripalmitin	112 ± 2	0	0	0	0
C^{14}-1-Leucine	131 ± 6	125 ± 4	106 ± 1	0	0
C^{14}-2-Glycine	184 ± 14	153 ± 9	128 ± 5	128 ± 6	128 ± 5
C^{14}-UL-Glucose	74 ± 4	83 ± 3	84 ± 3	85 ± 3	88 ± 2

* Data calculated from $BaCO_3$ collected from experiments recorded in Table I.
† No change from controls.

TABLE III. Effect of Age on Cumulative Oxidation of C^{14}-2-Acetate by Rats Given Either U-9189 or DNP.*

Group	% of C^{14} oxidized to CO_2 at: ½ hr	1 hr	2 hr	3 hr	5 hr
Young: Controls (6)	22.2 ± 2.0	42.1 ± 2.2	55.5 ± 3.0	57.2 ± 3.0	62.6 ± 3.4
U-9189 (6)	26.9 ± 1.7	48.7 ± 1.0	61.3 ± 1.5	64.1 ± 1.7	65.7 ± 1.7
DNP (4)	21.6 ± 3.3	41.8 ± 4.2	59.4 ± 1.1	60.8 ± 1.9	67.1 ± 1.2
Adult: Controls (6)	11.6 ± 1.6	26.7 ± 5.4	44.9 ± 6.7	51.9 ± 4.5	58.6 ± 5.3
U-9189 (6)	25.6 ± 1.5	44.8 ± 2.4	52.3 ± 2.2	56.1 ± 3.2	65.2 ± 5.1
DNP (4)	22.0 ± 1.9	44.6 ± 6.9	61.4 ± 5.2	65.2 ± 6.5	73.1 ± 7.0

* 50 mg/kg I.P. U-9189 or 15 mg/kg DNP increased C^{14} specific activity as well as total exhaled CO_2 only in adult rats.

TABLE IV. Oxidation of C^{14}-1-Leucine by Fasted Thyroidectomized Rats.*

	Injected C^{14} oxidized to $C^{14}O_2$									
	½ hr		1 hr		2 hr		3 hr		5 hr	
Group	% O‡	SA§	% O	SA	% O	SA	% O	SA	% O	SA
Control (6)	3.5 ±.5		13.7 ± 1.2		20.2 ± 1.9		25.5 ± 2.0		28.7 ± 2.4	
U-9189 (6)†	3.8 ±.4	108 ±1	16.6 ± 1.3	109 ±2	25.2 ± 1.5	0	30.0 ± 2.4	0	41.5 ± 3.5	0

* Effect of U-9189 was tested 2 wk after thyroidectomy in rats specially chosen (see *Methods*). Control values were obtained on these same rats 5 days previous to inj. of U-9189.

† U-9189 treatment increased body temperature and total CO_2 output 40% by the 5th hr.

‡ Cumulative % of C^{14} dose oxidized.

§ Specific activity (% of controls), 0 indicates no change from controls.

Oxidation by thyroidectomized rats. U-9189 increased the metabolic rate of fasted thyroidectomized rats also. Both cumulative $C^{14}O_2$ from C^{14}-leucine, as well as total CO_2, increased about 40% by the fifth hour (Table IV and footnote, Table IV). It is interesting to note that, in spite of a similar increase in CO_2, intact rats showed a greater increase in $C^{14}O_2$ specific activity during the first hour, *e.g.*, 25-31% (Table II), while operated rats showed only a slight increase of 8-9% (Table IV). Absence of thyroid gland therefore altered overall oxidative pattern. Presence of thyroid hormone (permissive action) for maximal response to reproductive hormones has been reviewed(7), but the nature of relationship between U-9189 and thyroid hormone suggested here awaits further experimentation.

Since the drug increased metabolic rate of thyroidectomized rats, it is unlikely that this action resulted from induced thyroid hormone secretion in intact rats. The mechanism whereby U-9189 increased metabolism *in vivo* was probably due to uncoupling of oxidative phosphorylation mentioned previously. However, U-9189 does affect the thyroid gland *per se* in that it can depress thyroid function as mentioned earlier. In view of this, it is interesting that a similar combined effect has been reported for salicylates. They have been shown to uncouple oxidative phosphorylation by a manner different from that reported for thyroxine(8) and to depress thyroid function(9). Salicylates have also been shown to release thyroxine from serum proteins(10) and it is possible that decreased C^{14} specific activity response obtained from thyroidectomized rats given U-9189 was related to lack of bound thyroid hormone which may have been released had it been present.

Summary. Administration of 1,1,3-tricyano-2-amino-1-propene (U-9189) increased total CO_2 output and oxidation of C^{14}-labeled acetate, tripalmitin, DL-leucine, and glycine in intact fasting rats. U-9189 also increased C^{14} specific activity of exhaled CO_2 suggesting increased utilization in excess of increased metabolic rate. Total oxidation of C^{14}-glucose was not influenced; resultant specific activity was therefore decreased. Although in thyroidectomized rats U-9189 produced a similar increase in total CO_2 expired and oxidation of C^{14}-DL-leucine, specific activity of total CO_2 increased only slightly compared to intact rats given U-9189. Total "oxidative effect" of U-9189 appeared to be lacking in the absence of thyroid gland.

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